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QUANTITATIVE CHROMATOGRAPHIC ANALYSES OF N-PHENYL-ALPHA-NAPHTHY--ETC(U)
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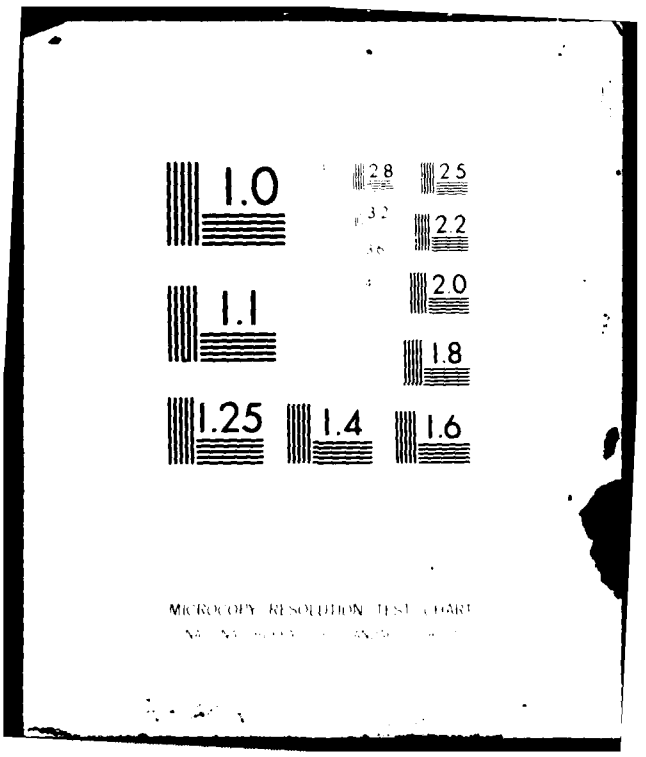
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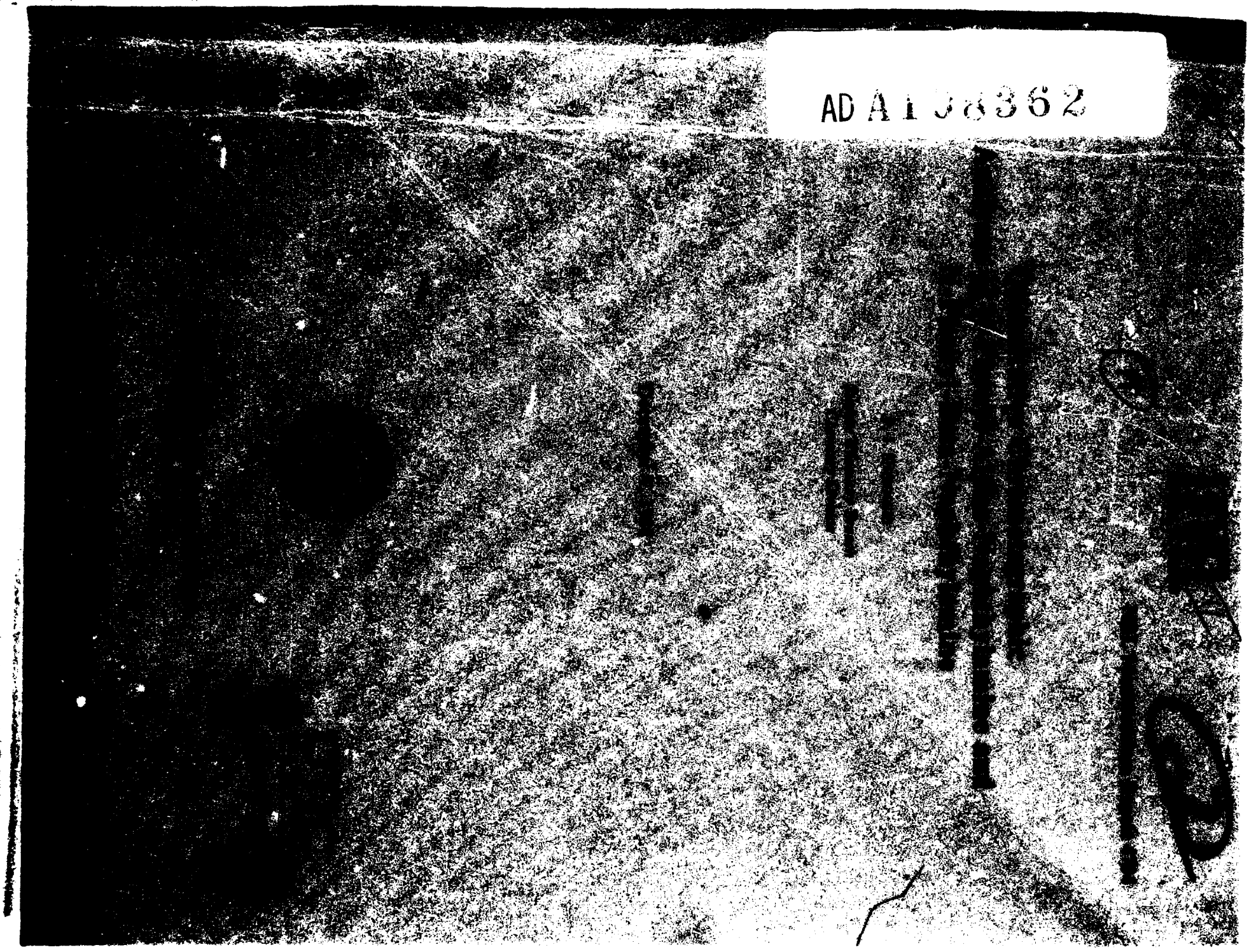

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Three convenient chromatographic methods were developed for the quantitative analysis of N-phenyl-beta-naphthylamine in samples of N-phenyl-alpha-naphthylamine. The TLC method makes use of dipropylene glycol impregnated silica gel plates. XE-60 is utilized in the GC method, and a column packed with NH_2 10 micrometers is used in the LC separation. Analyses were carried out on various commercial samples of N-phenyl-alpha-naphthylamine that were to be used as an antioxidant in aircraft engine lubricants.

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QUANTITATIVE CHROMATOGRAPHIC ANALYSES OF N-PHENYL-ALPHA-NAPHTHYLAMINE AND N-PHENYL-BETA-NAPHTHYLAMINE

INTRODUCTION

N-phenyl-alpha-naphthylamine (PAN) and N-phenyl-beta-naphthylamine (PBN), Figure 1, are widely used as antioxidants in products such as rubber and lubricants. Production of these antioxidants runs into millions of pounds annually. The use of PAN in military aircraft lubricants alone accounts for the annual consumption of more than one hundred thousand pounds. For many years these additives were handled as reasonably safe materials. In view of current, general concern of the effects of chemicals on health, they have been examined for possible mutagenic and carcinogenic activity (1-3). It was determined that in humans PBN is partially metabolized to beta-naphthylamine which has been associated with bladder cancer (4). Initiated in April 1981, the National Toxicity Program is supporting a two-year program of conducting PBN carcinogenicity tests on rats and mice. The Navy is interested in determining the levels of PBN in commercial samples of PAN that are to be used in the additive package of its aircraft engine lubricants.

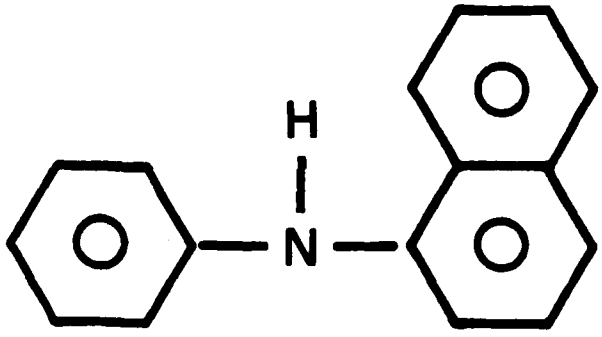
Although there are available a variety of chromatographic analytical methods for these widely used antioxidants (5,6), none of those investigated were suitable for the present purpose, primarily because the relatively large band of PAN obscures or overlaps the neighboring small band of PBN. In this present study, three simple and convenient methods are described that reduce this difficulty and quantitatively measure the presence of PBN in PAN down to the 0.1 percent level.

Manuscript submitted October 19, 1981.

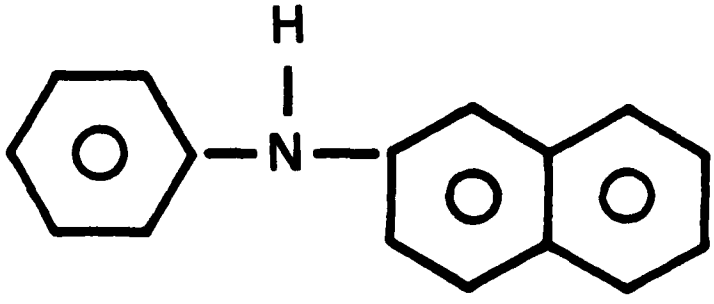
EXPERIMENTAL

Quantification of chromatographic methods

Measurements were made from recorder peak heights. Calibration samples in the range of 0.1 to 0.001 percent PAN and PBN were prepared in heptane and used throughout the analyses.



N-PHENYL-ALPHA-NAPHTHYLAMINE
PAN



N-PHENYL-BETA-NAPHTHYLAMINE
PBN

Figure 1

Thin-layer chromatography

E. Merc thin-layer glass plates, 10 x 20 cm, precoated with a 0.25 mm thickness of silica gel, were treated with a solution of 10 percent dipropylene glycol in acetone. After the acetone had evaporated, the plates were spotted with 1-microliter samples of heptane solution of the unknowns along with appropriate calibration samples. The plates were developed in heptane with a solvent rise of 7 cm. The R_f values were 0.48 for PAN and 0.31 for PBN. The freshly-developed plates were scanned with a Kontes Densitometer K-49-500 in the long wave UV fluorescence mode. Upon exposure to the air, the fluorescence begins to decrease fairly rapidly. However, the relationship of the measured peak heights of the samples measured at a single scan is not changed significantly.

Gas-liquid chromatography

Analysis was carried out at 240°C with a helium flow of 20 ml per minute through a stainless steel column 300 x 0.32 cm packed with 3 percent XE-60 on 80/100 mesh Chromosorb W. Sample size was 2 microliters of a heptane solution. The adjusted retention times were 2.0 minutes for PAN and 3.0 minutes for PBN.

Liquid chromatography

Use was made of 22 x 0.64 cm column of NH_2, 10 micrometers (Spectra physics) and a UV detector monitoring at 254 nm. With a flow rate of 3 ml of heptane per minute, the adjusted retention times were 8.5 minutes for PAN and 19.0 minutes for PBN. A summary of the various chromatographic methods is given in Table 1.

Solubility measurements

Solubilities of PAN and PBN in heptane, water, and pentaerythritol tetravalerate were determined. The heptane solubility was determined

Table 1. Summary of Chromatographic Separations of PAN and PBN

Method	Separation Parameters PAN	PBN
TLC - heptane development on dipropylene glycol impregnated silica gel plates.	0.48 R_f	0.31 R_f
GC - 300 cm column of 3% XE-60 at 240°C.	2.0 min.	3.0 min.
LC - 22 cm column of NH_2 10 micrometers (Spectra physics) with heptane mobile phase.	8.5 min.	19.0 min.

gravimetrically. For the water solubility determination, sonic agitation and warming were utilized in the preparation of the solutions. The samples were allowed to stand for several days and then filtered through an 80-micron filter. Analysis by liquid chromatography was carried out on heptane extracts of the water solutions. In the case of the solubility in pentaerythritol tetravalerate, heated saturated solutions were allowed to cool and stand for a week before analysis. After the samples were passed through an 80 micron filter and appropriately diluted with heptane, analysis was carried out by liquid chromatography. Solubility results are given in Table 2.

Determination of PBN in PAN samples

Chromatography of the PAN samples was carried out on two percent solutions in heptane. The chromatographed unknown and calibration samples were of equal size, so that values for the PBN content were based on the calculation

$$\text{\% PBN in the PAN sample} = \frac{50\ (\text{PBN peak ht. in PAN sample})(\text{\% PBN in calibration sample})}{(\text{PBN peak ht. in calibration sample})}$$

Results of the analyses by the three methods are given in Table 3. For a typical value of 0.1% PBN in the PAN sample, the standard deviation was of the order of 0.01. Figure 2 shows chromatograms of a sample of PAN containing 0.05 percent PBN.

Mass Spectra

Mass spectra were obtained from the residues of manually collected fractions from the liquid chromatograph and placed in the solid probe of a Model 9500 Finnigan Mass Spectrometer operated at 70 eV. Fig. 3 shows a partial spectra of the residue from the suspected PBN peak collected in 5 ml of effluent from Sample 4 listed in Table 3 and the residue from 5 ml of effluent collected between the PAN and PBN peaks of this sample. Also shown is the spectra of an authentic PBN sample.

Table 2. Solubility Data for PAN and PBN at 23°C

	Heptane (%)	Water (ppm)	Pentaerythritol-tetravalerate (%)
PAN	3.49	3.7	43.0
PBN	0.55	0.24	14.1

Table 3. Percent PBN in PAN Samples

		Method of Analysis		
No.	Sample	LC	GC	TLC
1	Reagent Grade	0.10	0.10	0.16
2	Reagent recryst.	0.004	0.005	-
3	Commercial	0.24	0.29	0.32
4	"	0.083	0.11	0.12
5	"	0.13	0.09	0.15
6	"	0.27	0.24	0.20
7	"	0.25	0.17	0.25
8	"	0.003	0.002	-

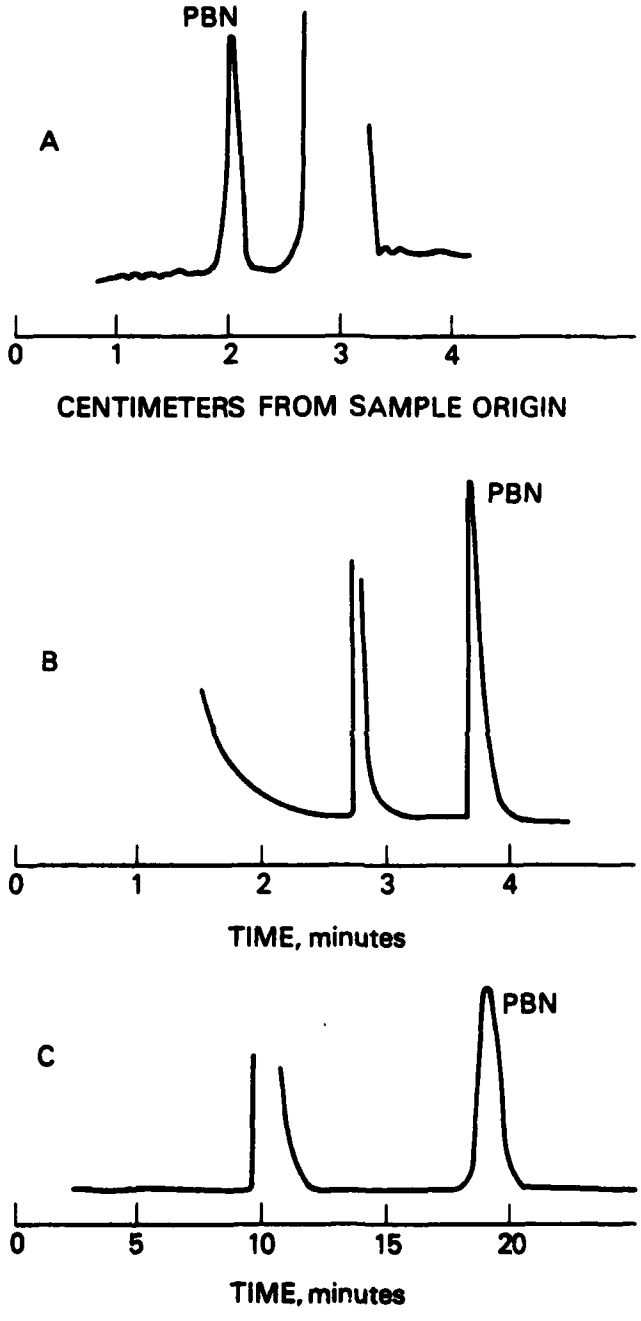


Fig. 2 — Chromatograms of 0.05% PBN in PAN: (A) TLC, (B) GC, and (C) LC

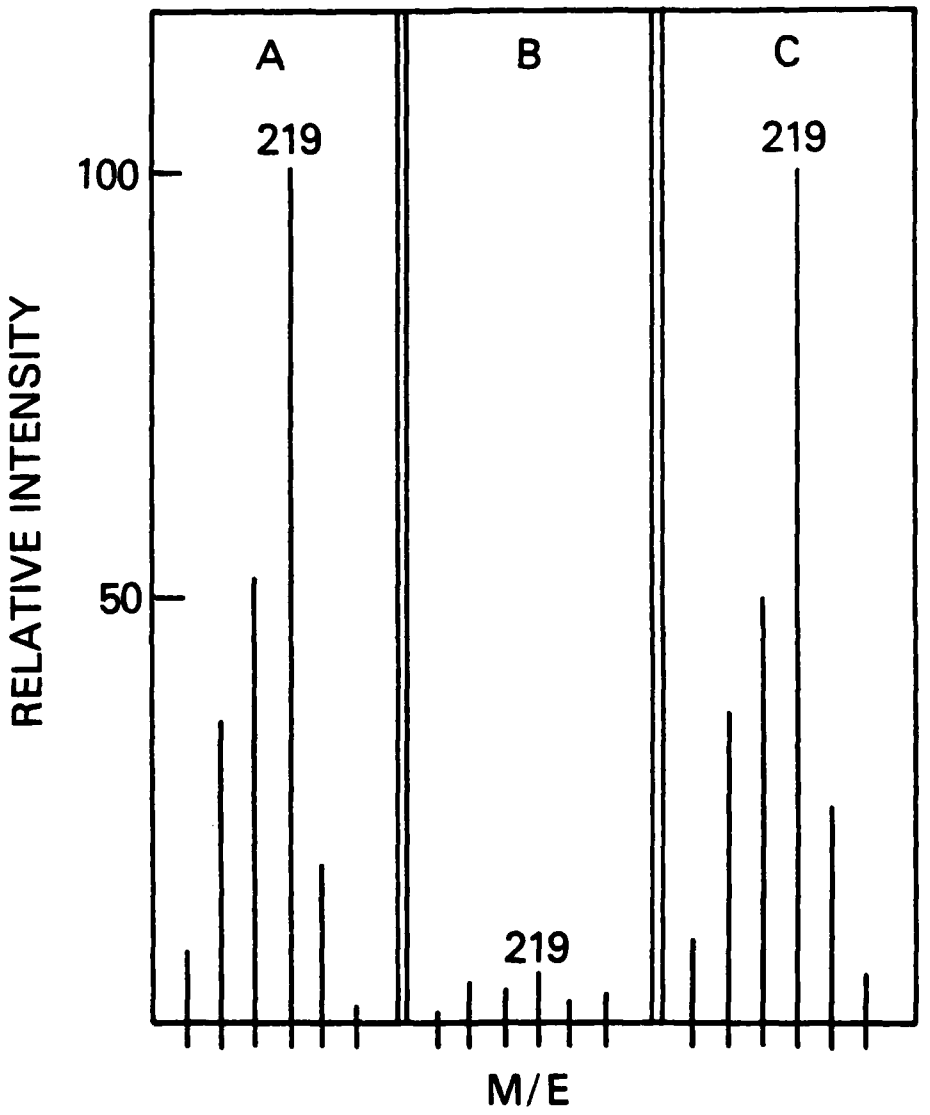


Fig. 3 — Partial mass spectra from (A) an authentic PBN sample, (B) LC effluent residue collected between PAN and suspected PBN peaks from Sample 4, Table 3, and (C) suspected PBN LC peak from the same sample

DISCUSSION

A cursory examination of the structures of these two isomers, shown in Fig. 1, indicates that the chromatographic properties should be quite similar. The work of Shimiza (7) with eight different chromatographic systems showed that the partition coefficient, defined as the ratio of the amount of sample in the more polar phase to that in the less polar one, is greater for PBN than for PAN. Therefore, it would be of some advantage to develop a chromatographic method where the PBN impurity eluted first, since it would not be overlapped by the tailing of the relatively large PAN band. However, as summarized in Table 1, PAN elutes before PBN in all three of the chromatographic systems. The separation factors in the methods are large enough to essentially eliminate interference of the PAN band.

In the course of developing these chromatographic systems, solubilities in heptane, water, and pentaerythritol tetravalerate, a typical ester component in aircraft lubricants, were determined and reported in Table 2. As expected, the lower melting isomer, PAN at 62°C, has a greater solubility than the higher melting PBN at 108°C. The value for the water solubility of PAN agrees with that of Greenhouse (1) who utilized a spectrometric method for analysis. Both isomers have considerable solubility in the pentaerythritol ester.

Commercial samples of PAN destined for use in the additive package of aircraft engine lubricants, along with a reagent sample of PAN, were analyzed for PBN content by all three of the chromatographic methods. Analyses were carried out on two percent solutions of the PAN samples in heptane with the aid of appropriate calibration standards. The results are given in Table 3, where it is seen that the PBN content is rather low. From a comparison of the results from Samples 1 and 2 of Table 3, it is demonstrated that recrystallization from heptane would be an effective method for removing the PBN impurity. Examination

of the analytical results in Table 3 reveals no significant difference in the methods. However, the LC and GC methods are capable of detecting PBN below the 0.1 percent level with reasonable reliability.

Mass spectra were determined in manually collected LC fractions to further substantiate the presence of the small amounts of PBN in the commercial samples of PAN. A complete verification was not feasible because of several factors. Not only is differentiation of the two isomers difficult because of the similarity of their mass spectra, but also the hydrocarbon residue in the solvent obscured a portion of the mass spectra. In the example shown in Fig. 3, derived from Sample 4 of Table 3, only the portion of the spectra 216 to 221 m/e is shown which is relatively unaffected by the solvent residue. In this range fall the three principal ions from PBN and also from PAN: the molecular ion 219 m/e and the ions 217 and 218 m/e. Also included is the isotope ion of the molecular ion at 220 m/e. Since the level of ions from PAN and/or PBN is so low in spectra B, collected between the PAN and the suspected PBN peaks, it is reasonably concluded that the ions present in spectra C are essentially produced by PBN.

CONCLUSIONS

Three chromatographic methods were described for the analysis of N-phenyl-naphthylamine in samples of N-phenyl-alpha-naphthylamine. These methods allow for a considerable overload of the N-phenyl-alpha-naphthylamine in the chromatographic system without a sacrifice of the quantification of the other isomer. Commercial samples of N-phenyl-alpha-naphthylamine that were to be used in the additive package of Navy gas turbine engine lubricants showed small amounts of the N-phenyl-beta-naphthylamine. Of the three methods developed in this report the LC one is preferred because of its relative ease of operation. The chromatographic column has been satisfactorily used over a period of three

years for the separation of these components and other amine antioxidants (8).

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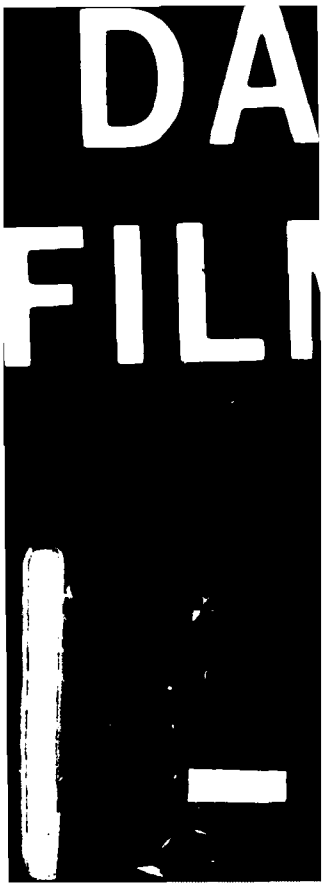
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